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Partitioning of D.L. Alanine and Hexaglycine in Dextran + Poly (ethylene glycol) + Water Two-Phase System at 297.15K

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ABSTRACT

Partitioning of two amino acids (DL-alanine and hexaglycine) were measured in dextran + poly(ethylene glycol)+ water two-phase system. The coefficient for partition of DL-alanine and hexaglycine were correlated by using the osmotic viral equation. The phase interaction or partitioning coefficientin the equation can be calculated by hydrophilic group's parameters which gives in fairly good agreement with the experimental data on change in concentration at 293.15Kat room pressure.

Keywords: Partition coefficient, Hydrophilic parameter, Osmotic Viral equation, Dextran.

INTRODUCTION

A predictive method for the partition coefficients (Kp) of several amino acids[1,15] in the dextran [DEX] poly(ethylene glycol)[PEG] + water aqueous two phase system has been proposed(Zhou *et al*. 1997) by using the osmotic virial equation(Edmand and Ogston, 1968; King *et al*.1988). Salt effect on charged protein partitioning[13] in aqueous PEG+ Dex system by potential (non electrostatic from electrostatic effect) proposed by Weiyu Fan and Charles E. Glatz (1999). The liquid-liquid interaction also helps in salting out effects[3,4,5]. There the viral coefficient needs in calculation of Kpvalues were given by the hydrophilic parameters[9,10]. The hydrophilic group parameters helps in pharmaceutical industry for recovery with density behavior[6,7]. It is of interest to extend this procedure to larger molecules such as peptides. In this study Kp values for two peptides (hexaglycine and DL alanine) in the same aqueous two phase

system were measured to determine new hydrophilic group parameters[5,6] required for particles behavior and their solubility and salting out effect[13] in two phase system. Prediction performance of the present method for Kp values of[11] peptide (hexaglycine and DL alanine) in PEG + DEX + water is presented and discussed.

EXPERIMENTAL

Dextran (DEX) was purchased from Merck as DEX T 40(MN=18800, mw=38000) and DEX T 500(MN=170300, MW=503000). Poly ethylene glycol (PEG) standard samples with two different molecular weights were purchased from E. Merck Pure Chemical Ind. as 200(MN= 194, mw=194) and PEG600(MN= 550, MW=580). DL-alanine and hexa-glycine were purchased from Sigma Chemical Company. All reagents were used A R grade without further purification. Triple distilled degased water was purified with a Millipore Milli-Q system in acidify KMnO4 solution.
Phase interactions of DL Alanine and Hexaglycine were carried out in thermostat at 293.15K. For constant temperature water was rotated by continuous starring. Reaction flask was fitted within the thermostat. Preparation of aqueous two- phase systems were prepared in triple distilled water with concentration range 0.01 $molL^{-1}$ to 0.10 mol L^{-1} for stand for 24 hr. to avoid any precipitation. The aqueous two- three component solution were kept into thermostat for 15min for constant temperature. The 1X 10^{-3}M solution of PEG`s (200and 600), Dextran T(40 and 500)were used for salting out. These concentrations were again measured. The liquid-liquid equilibrium for PEG`s and Dxetran has recently been investigated. In order to determine the partition co-efficients of the DL-Alanine and Hexaglycine in liquid system, two tie line were selected from each phase diagram. Presentation of the two phase samples were performed in 20 ml graduated glass bottles tightly closed. The total wt. of the component was about 7.5gm. The initial wt. of the DL-alanine and Hexaglycine added to system was $0.5X10^{-2}$ gm. The mixtures were shaken for 20 min. and then placed in thermostatic water bath for at least 24hr. to ensure complete equilibrium. Syringes were used for samples approximately 5ml carefully removed for analysis from the top and bottom layer. The separated solutions were centrifuge for 2 min. and the uncertainty in the amino acid concentration for analysis of top and bottom phase was less than ±1.8 ml/L.

RESULTS AND DISCUSSION

The partition coefficients of the two amino acids(DL-alanine andhexa-glycine) in DEX T40 + PEG200 + water, DEX T500 + PEG200 + water, DEX T40 + PEG600 + water and DEX T500 +PEG600+ water aqueous two phase systems are presented in Table 1,and concentration ranges were 1.0-2.0 mili mole/ litre. The partition coefficients values were calculated w.r.t. tie line lengths at particular concentration. These data were obtained from an arithmetic average of at least three –four measurements for each condition. The some unequal distributions of these small molecular compounds especially for amines with lower ratio were established despite the experimental errors. The TLL (tie-lines Length) data of the same aqueous two-phase systems have been reported elsewhere

(Furuya *et al*., 1995a, 1996).

The partition coefficient, kp, and tie –line length are defined, respectively, as follows.

$$TLL = [(W1 - W2) + (W1 - W2)]^{1}/^{2}$$

$$Kp = \frac{The\ conc.of\ amino\ acids\ in\ PEG\ rich\ phase}{The\ conc.of\ amino\ acid\ in\ DEX\ rich\ \ top\ phase}\text{mg/g}$$

where W is the weight fraction and subscripts 1 and 2 indicate DEX and PEG, respectively. The accuracy of the experimental partition coefficient data is considered to be within ± 5% from the reproducibility.

From Table 1, it can be seen that Kp of DL-alanine and hexa-glycine decrease with increasing tie-line length and Kp of DL-alanine are reported to be smaller than those of hexa-glycine. At lower concentration of DL-alanine and hexa-glycine in DEX T40 + PEG200 + water were large difference in their Kp values due to larger difference in TLL (Table1), it is shown that solvent –solute interaction are stronger than that of solvent-solvent interactions, which correlated Kp values with the more hydrophilic groups (Table 3). : Effect of conc. on location of binodal curve at 293.15K (Table 2) were reported for PEG 200 and 600 with DL-alanine and hexaglycine.. As wt% for PEG 200 decreases from 39.12-30.00, increase wt% for DL alanine and hexahlycine were reported to 2.55 and 1.98, for 600 decreases from 41.23- 28.92 were 2.52 and 2.49. These effects on salt distribution are the result of change in solvent structure and of polymer-salt (Zwitter ion) and polymer-polymer interactions. Long chain of peptide bond will reduce PEG solubility in the DEX phase and amino acid solubility in the PEG phase, which leads to a more uneven distribution of polymers and amino acid between two phases.

Interaction Parameters of Osmotic Virial Equation

To predict of the KP values, the osmotic virial equation proposed by Edmond and Ogston (Edmond and Ogstomn, 1968; King *et al*. 1988) can be applied. The partition coefficient in the infinite dilute condition can be derived as:

$$\frac{lnKp}{m_2 m_2} = a_4\left[\frac{m^1 m^1}{m^2 m^2}\right] + a_4$$

by plotting lnkp(m)/ (m2BOT-m2TOP) vs. (m1 BOT-m1TOP)/(m2 BOT-m2TOP), the interaction parameters a14 and a24 can be determined. The partition coefficient, Kp(m), expressed by the ratio of molalities can be transformed as follows by the experimental results of Kp,

$$Kp\frac{m_4}{m_4} = \left[\frac{1000 + M_1 m_1 M_2 m_2}{1000 + M_1 m_1 M_2 m_2}\right]$$

where η is the hydrophilic parameter and is defined as follows:

$$\eta = \frac{\Sigma^{-1}\eta kNk}{\begin{array}{c}the\ number\ of\ total\ atoms\ of\ amino\\ acids\ or\\ peptide\ except\ hydrogen\end{array}}$$

the number of total atoms of amino acid or peptide except hydrogen in equation, nk is the hydrophilic parameter of group k, and Nk is the number of group k contained in amino acid or peptide. The authors used the

same values of ηi4 and βi4 (i=1, 2) proposed by Zhou *et al*. (1997) and determined the new values of nk by the data of Kp for salts and peptides obtained in this work (Table 2). The values of nk determined by Zhou *et al*. (1997) and obtained additionally in this work are shown in Table 3. Table 4 shows the deviations between experimental and calculated K p for salts or peptides.

Table 1: Experimental. Partition coefficients of D L Alanine and Hexa-glycine in aqueous two phase at 293.15 K.

Aqueous two phase	Concentration Mol L^{-1}	Tie- line Length Wt %	Kp	
			DL Alanine	Hexa-glycine
DEX T 40 + PEG 200 + Water	$1.0X\ 10^{-3}$	13.33	0.84	0.83
	$1.3X\ 10^{-3}$	18.16	0.74	0.69
	$1.7X\ 10^{-3}$	21.98	0.69	0.65
	$2.0X\ 10^{-2}$	24.84	0.68	0.63
DEX T 40 + PEG 600 + Water	$1.0X\ 10^{-3}$	08.53	0.90	0.84
	$1.3X\ 10^{-3}$	09.15	0.81	0.87
	$1.7X\ 10^{-3}$	10.48	0.78	0.63
	$2.0X\ 10^{-2}$	11.37	0.73	0.62
DEX T 500 + PEG 200 + Water	$1.0X\ 10^{-3}$	11.94	0.85	0.84
	$1.3X\ 10^{-3}$	13.33	0.80	0.81
	$1.7X\ 10^{-3}$	17.00	0.77	0.76
	$2.0X\ 10^{-2}$	21.98	0.72	0.65
DEX T 500 + PEG 600 + Water	$1.0X\ 10^{-3}$	06.62	0.89	0.86
	$1.3X\ 10^{-3}$	09.56	0.80	0.78
	$1.7X\ 10^{-3}$	12.48	0.79	0.76
	$2.0X\ 10^{-2}$	14.48	0.74	0.69

Table 2: Effect of conc. on location of binodal curve at 293.15K

PEG200 Wt%	DL Alanine Ws%	Hexa-glycine Wt%	PEG600 Wt%	DL Alanine Ws%	Hexa-glycine Wt%
39.12	10.50	9.85	41.23	9.00	7.51
38.90	11.32	9.96	38.44	9.23	7.94
38.10	11.85	10.22	36.45	9.82	8.12
37.15	12.15	10.45	34.65	10.10	8.45
36.56	12.45	10.87	33.05	10.55	8.78
34.67	12.82	11.05	31.51	11.24	9.11
32.35	12.95	11.48	30.15	11.28	9.35
30.00	13.05	11.83	28.92	11.52	10.00
28.85	13.40	12.25	27.80	11.74	10.30

Table 3: Hydrophilic group parameters ηn at 303.15K (Reference values)

Group K	OH Alcohol	ACH	C-	NH	CHn	CONH	COOH	NH2	O-	N-
ηK	0.949	0.104	O.O79	0.062	0.0	2.3	0.75	0.432	0.57	1.13

Table 4: % Deviation's for Kp values

Systems	DL Alanine	Hexa –glycine
PEG 200+water	2.8	2.9
PEG 600+ water	3.5	2.2
DE X T-40+ Water	4.4	3.8
DEX T 500+ Water	4.5	4.7
DEX T-40+PEG 200+water	4.8	7.3
DEX T-40+PEG 600+ water	4.9	3.8
DEX T-500+ PEG 200+ Water	6.3	3.1
DEX T-500+ PEG 600+ Water	5.1	1.3

CONCLUSION

The partition coefficients of two peptides hexaglycine and DL- alanine in dextran+ poly (ethylene glycol) + water aqueous two phase systems were measured. The partition coefficients of two amino acids (DL alanine and hexaglycine) obtained in this study was correlated by using the osmotic virial equation. The interaction coefficients (Kp) are estimated by using hydrophilic group parameters and successful correlation and results are obtained. The tie line lengths/ composition were correlated by hydrophilic group parameters and salting out ability. Furthermore, by using the same procedure, the partition coefficients of amino acids are predicted show fairly good agreement.

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